The Aryl-hydrocarbon Receptor (AhR) as a Therapeutic Target in Human Breast Cancer

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Abstract

Breast cancer is currently the most prevalent malignancy among women in industrialized countries, and 1 in 8 women living in the United States will develop the disease at some point in her lifetime. Although the incidence of breast cancer has increased 0.3% per year since 1990, the mortality rate has decreased by 2% per year since 1990 due to improvements in treatment and early detection. New treatment strategies and a better molecular understanding of the disease will be important to continue the progress science has made against this disease.

The aryl-hydrocarbon receptor (AhR) has been traditionally associated with activation by environmental contaminants, acute toxicity, and cancer risks associated with exposures. However, the AhR has been highly conserved throughout evolution suggesting an important biological role for the receptor independent of its response to environmental contaminants. There is a significant body of evidence indicating the AhR plays a role in breast epithelial cell differentiation and that receptor agonists can inhibit breast cancer growth. A 50% reduction in estrous-induced terminal end buds was observed in the mammary glands of AhR knockout animals when compared to wild type suggesting a role for the AhR in mammary development. In 2 independent rodent cancer bioassays, treatment with the AhR agonist 2,3,7,8-tetrachlorodibenzo-pdioxin (TCDD) significantly reduced the incidence of spontaneous rat mammary tumors. These and other studies support the premise that the AhR plays a fundamental role in breast epithelial cell differentiation. This review provides a brief summary of the current evidence that the AhR may be an important pharmacological target for treating human breast cancer.

Introduction

Breast cancer is currently the most prevalent malignancy among women in industrialized countries, and 1 in 8 women living in the United States will develop the disease at some point in her lifetime [1]. Although the incidence of breast cancer has increased 0.3% per year since 1990, the mortality rate has decreased by 2% per year since 1990 due to improvements in treatment and early detection [1]. New treatment strategies and a better molecular understanding of the disease will be important to continue the progress science has made against this disease.

The aryl-hydrocarbon receptor (AhR) has been traditionally associated with activation by environmental contaminants, acute toxicity, and cancer risks associated with exposures. However, the AhR has been highly conserved throughout evolution [2] suggesting an important biological role for the receptor independent of its response to environmental contaminants. There is a significant body of evidence indicating the AhR plays a role in breast epithelial cell differentiation and that receptor agonists can inhibit breast cancer growth. A 50% reduction in estrous-induced terminal end buds was observed in the mammary glands of AhR knockout animals when compared to wild type suggesting a role for the AhR in mammary development [3]. In 2 independent rodent cancer bioassays, treatment with the AhR agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) significantly reduced the incidence of spontaneous rat mammary tumors [4,5]. These and other studies support the premise that the AhR plays a fundamental role in breast epithelial cell differentiation. This review provides a brief summary of the current evidence that the AhR may be an important pharmacological target for treating human breast cancer.

The Aryl-Hydrocarbon Receptor (AhR) Mechanism of Action

The identification of the AhR originated in early toxicology studies that observed an increase in mono-oxygenase activity following exposure to polyaromatic hydrocarbons. Additional studies using a highly potent inducer, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), showed that binding to a cytosolic receptor was proportional to the induction of mono-oxygenase activity [6]. The gene encoding the AhR was subsequently cloned and characterized across multiple species [7-9]. Further molecular characterization of the AhR demonstrated that the receptor is highly conserved [2,10] and plays a significant role in tissue development [1,11-13]. For example, targeted disruption of the AhR in the mouse results in compromised immune function [14], and deficiencies in the development of several tissues including ovarian follicles [15], seminal vesicles [16], fetal vasculature [17], and mammary glands [18].

The AhR is a ligand-activated transcription factor and a member of the basic-helix-loop-helix Per-Arnt-Sim (bHLH-PAS) family of proteins [19]. In an unliganded state, the AhR exists in the cytoplasm

bound to chaperones HSP90, p23, and other proteins (Figure 1). Ligand binding induces translocation of the AhR into the nucleus, where it dissociates from cellular chaperones and heterodimerizes with ARNT (aryl-hydrocarbon receptor nuclear translocator). AhR/ARNT heterodimers bind to genomic dioxin response elements (DREs), which initiates transcription of genes within the AhR gene battery, including Cyp1a1, Cyp1b1, Aldh3, Nqo1, and Gsta1 [20-23]. Interactions with other cellular proteins have been identified leading to a proposed role for the AhR in many signaling pathways including the retinoblastoma (Rb) protein and cell cycle [24,25], estrogen receptor (ER) signaling [26] and RELA and NFκB signaling [27].
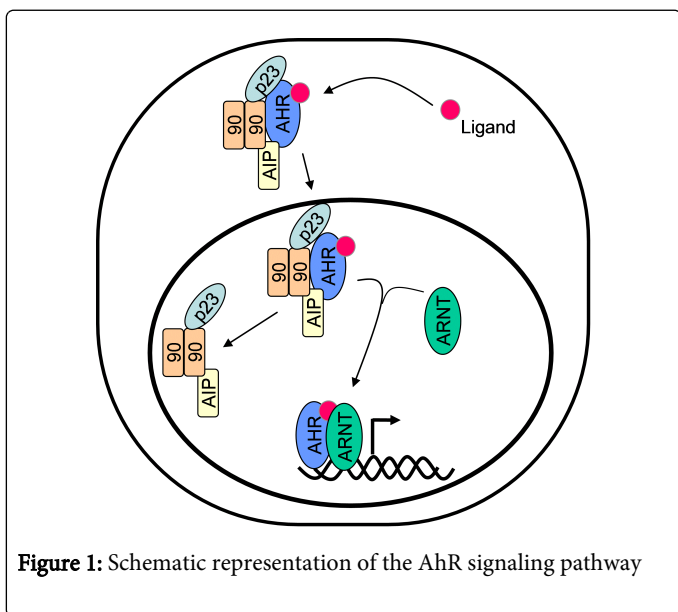

Figure 1: Schematic representation of the AhR signaling pathway

Role of the AhR in Tissue Development

The AhR gene is highly conserved across both vertebrate and invertebrate species [2,10]. The existence of ancestral AhR orthologs that either bind no ligand or bind a range of ligands that are unique from those recognized by the vertebrate receptors suggest that its role in regulating xenobiotic metabolism is a recent adaptation [2,28]. Apart from the evolutionary conservation, evidence for a role of the AhR in tissue development can be seen from the characterization and disruption of the AhR in model organisms. In Drosophila melanogaster, mutation of the AhR ortholog Spineless lead to alterations in appendage development [29], neuron morphology [30], and photoreceptor development [31].

In Caenorhabditis elegans, disruption of the AhR ortholog AHR-1 leads to alterations in neuronal development [32]. In a more relevant mammalian model, expression of the AhR occurs early in development in preimplantation embryos [33] and at gestational days 10 to 12 with high expression in many neuronal tissues [34]. Expression of the AhR expands to a large number of tissues by gestational day 13.5 to 15.5 indicating a potentially broad functional role in tissue development [34]. Targeted disruption of the AhR in mice has revealed obligatory roles for the receptor in multiple tissues including liver, the immune and cardiovascular systems, and the male and female reproductive systems [14-18]. Notably, a 50% reduction in estrous-induced terminal end buds was observed in the mammary glands of AhR null animals when compared to wild types suggesting a role for the AhR in mammary development [3].

Ligands of the AhR

Following the early studies characterizing the increase in mono-oxygenase activity due to polyaromatic hydrocarbon exposure, a significant research effort has ensued to identify and characterize both endogenous and xenobiotic ligands for the AhR. Although the endogenous ligand for the AhR is the subject of continued debate, multiple candidates have been proposed including indigoids, equilenin, arachidonic acid metabolites, heme metabolites, tryptophan metabolites, and UV photoproducts of tryptophan [35]. In addition, dietary compounds and nonligand activators of the AhR have been identified [35]. For the xenobiotic ligands, the most well-known are the halogenated dioxins, polychlorinated biphenyls (PCBs), and polyaromatic hydrocarbons (PAHs) [20].

The halogenated dioxins include the dibenzo-p-dioxins and dibenzofurans that are formed during both combustion and industrial processes such as waste incineration, forest fires, pesticide manufacturing, and paper pulp bleaching [36]. Within this class, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDBF) are prototypical AhR agonists due to their potency and efficacy in receptor activation [20]. For the PCBs, the coplanar congeners with adjacent halogens in the lateral positions of each ring and no halogen atoms near the biphenyl bridge are the most potent AhR agonists [37]. These chemicals are used in a variety of commercial products including flame retardants, adhesives, transformers and capacitors, lubricants, and fluorescent light ballasts [38]. Finally, the PAHs are a large class of AhR agonists that contain four or more benzene rings [39]. Prototype AhR ligands in this category include benzo[α]pyrene and 3-methylchloranthrene. PAHs are found as byproducts of combustion processes and are typically much less potent than TCDD or the coplanar PCBs.

Adverse Responses to AhR Ligand Activation

The effects of AhR activation have been primarily characterized using xenobiotic ligands such as TCDD, PCBs, and PAHs. For the classical AhR agonist TCDD, sufficient doses can cause cancer, immune dysfunction, wasting, chloracne, ovarian failure, and birth defects [20,40-42]. Both AhR null animals and mice with a mutation in the DNA binding domain of the AhR are refractive to the toxic effects of TCDD, suggesting that the AhR and its binding to DNA are required to these effects [43,44]. For human cancer, epidemiological data from occupationally exposed workers have established a clear association between exposure to TCDD and increased risk for all combined cancers and for lung cancer [45]. The magnitude of the increased risk in these studies is generally low, but it is higher in sub-cohorts considered to have the highest exposure to TCDD [45]. In the rat, three separate bioassays have been performed [4,5,46].

In the most recent bioassay by the National Toxicology Program, an increased incidence was observed for cholangiocarcinomas and hepatocellular adenomas of the liver, cystic keratinizing epithelioma of the lung, gingival squamous cell carcinoma of the oral mucosa, and squamous cell carcinoma of the uterus [5]. There is substantially less information regarding potential adverse responses for potential endogenous or dietary ligands of the AhR with the majority of exposure occurring for relatively short time periods. Exposure of up to 50 mg/kg of indigo or indirubin per day for a period of 3 days had little effect on the rat liver [47]. Similarly, exposure mice to 20 mg/kg of equilenin per day for 3 days had no reported effects [48]. Longer exposures have been performed for indole-3-carbinol (I3C) (a

component of cruciferous vegetables) and its metabolite diindolylmethane (DIM). In exposures lasting up to one year, there were no observable differences grossly or histologically between groups and no changes in blood chemistry except that male rats treated with high doses of I3C exhibited higher serum levels of 25-hydroxy-vitamin D3 [49].

Protective Role of the AhR in Breast Cancer

While historically AhR agonists have been viewed as tumor promoters, combined epidemiological, animal bioassay, and molecular data indicate that this is an oversimplification. Examination of a population exposed to TCDD following an industrial accident in Seveso, Italy in 1976 showed a positive correlation between TCDD exposure and sarcomas, cancers in the prostate, lung, bladder and digestive tract, those in hematopoietic and lymphatic tissues, and all cancers combined [50], supporting previous studies of occupational workers [45]. However, a significant decrease in breast and endometrial cancer was also evident in exposed individuals [50]. More recently, a report following a population exposed to dioxin emissions from a municipal waste incinerator noted a significant decrease in incidence of invasive breast cancer in women living in the highest exposed zone [51].

The reduction in breast cancer observed in the human studies is supported by two independent rodent experiments showing a significant decrease in spontaneous mammary tumor incidence following a two-year exposure [4,5]. In an initiation-promotion model, an absence of mammary tumors was observed in rats administered TCDD compared with a 36% incidence in control animals [52]. Similarly, exposure to TCDD at sub-toxic doses decreased 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumors by 72% and, in a subset of animals, the original tumors were no longer detectable [53]. In contrast, control animals displayed a 390% increase in tumor volume over the same time period. Finally, in animals harboring ER+ human breast cancer cell xenographs, TCDD was found to completely suppress hormone-stimulated tumor growth [54]. These results suggest that, similar to many nuclear receptor agonists, the underlying biological responses to AhR activation are context specific.

Cross-Talk between the AhR and Estrogen Receptor α (ERα) Signaling Pathways

Cross-talk between the AhR and ERα signaling pathways occurs at multiple levels and is dependent on cellular context. In the promoters of many estrogen-inducible genes (e.g., pS2, cathepsin D, c-fos), binding sites for the AhR-ARNT complex (DREs) are adjacent to or overlap those of the ERα providing a means for the direct transcriptional inhibition of ERα [55-57]. The AhR-ARNT complex can also compete with ERα for coactivators, thereby dampening the transcriptional response [58]. On a post-translational level, liganded AhR can antagonize estrogen signaling by facilitating assembly of an ubiquitin ligase complex that promotes proteasomal degradation of ERα [59]. Finally, the AhR gene battery includes the cytochrome p450s Cyp1a1 and Cyp1b1 [20-23]. Upregulation of these enzymes results in an increase in estrogen metabolism and depletion of hormone levels [60,61].

The functional consequences of the antagonistic AhR-ER cross-talk are apparent in breast cancer cells. In growth assays, TCDD was shown to completely reverse the proliferative effects of estrogen in human breast cancer cells [54,62]. These observations have been recapitulated in vivo. In a nude mice xenograft model, TCDD suppressed estrogen-dependent tumor growth [54] and in a rat mammary tumor model, co-treatment of an AhR ligand with the antiestrogen tamoxifen was shown to completely inhibit growth of carcinogen-induced mammary tumors [63].

The Effects of AhR on Proliferation and the Cell Cycle

Apart from antagonistic effects on ERα signaling, the AhR may also display protective effects against human breast cancer by regulation of key processes required for breast cancer cell growth, cell cycle control, and cell migration. A number of studies have investigated the role of the AhR in cancer cell proliferation. Paradoxically, the AhR appears to facilitate growth in the absence of ligand, while the agonist-activated receptor displays marked anti-proliferative effects in cancer cells. For example, transfection of breast cancer cells with AhR siRNA is growth-inhibitory [64,65]. Similarly, AhR-deficient hepatoma cells (AhR-D) proliferate at a slower rate than wild-type cells (Hepa 1c1c7), and ectopic expression of the AhR in the AhR-D cells increases their growth to the rate of wild-type cells [66]. In contrast, a ligand-bound AhR inhibits cell growth in a wide array of breast cancer cell lines representing the major breast cancer subtypes, including both those positive and negative for ER, Progesterone Receptor (PR), and Human Epidermal Growth Factor 2 (HER2) [3,62,67-70].

It is now apparent that AhR-mediated suppression of cell growth involves G1 cell cycle arrest [25,71-74]. The cell cycle arrest coincides with association of the AhR with the hypophosphorylated and active form of Rb to form transcriptional repression complexes on several E2F-regulated genes required for S-phase progression [24,25]. In the absence of ligand, however, the AhR facilitates cell cycle progression by forming a complex with CDK4 and Cyclin D1 [74]. Thus, AhR agonists may function as a molecular switch in cancer cells, converting a basal AhR with growth-promoting activities into a potent anti-proliferative factor.

Evidence for a Protective Role of the AhR in Breast Cancer Cell Metastasis

In addition to cell growth and cell cycle regulation, the AhR has been shown to regulate genes involved in metastasis. Breast tumors most often metastasize to the lung and bone. These organs produce and secrete a chemokine, CXCL12, which attracts breast cancer cells expressing its receptor, CXCR4, on their cell surface [75]. In a recent study, TCDD was shown to down regulate both CXCR4 and CXCL12 in a breast cancer cell line and decrease cell migration towards a CXCL12 gradient [76]. While the consequences of CXCL12 suppression in these cells are unclear, the study demonstrated TCDD may be adequate in preventing breast cancer cells from migrating to areas with high CXCL12 expression [76].

There is emerging evidence that ligand activation of the AhR can inhibit multiple aspects of the metastatic process. In a panel of breast cancer cell lines that represent the 3 major breast cancer subtypes (ER and PR-positive, HER2-positive, and triple-receptor negative), it was found that agonist-activated AhR inhibited cell invasiveness and motility and prevented anchorage-independent growth [65]. Knockdown of the AhR with siRNAs demonstrated that the inhibition of invasiveness was receptor-dependent and endogenous receptor activity was protective in each cell type examined [65]. Likewise, AhR agonists were found to promote differentiation of breast cancer cells

and mammary cancer stem cells into cells that displayed the phenotypic markers of normal breast epithelial gland cells [65]. Thus, the AhR plays an important role in mammary epithelial differentiation and, as such, represents a promising therapeutic target for a range of phenotypically distinct human breast cancers.

The AhR as a Potential Therapeutic Target for Human Breast Cancers

Analogous to the selective estrogen receptor modulator (SERM) concept, it has been proposed that selective AhR modulators (SAhRMs) exist that are capable of activating the specific signaling pathways involving the protective effects of the AhR in breast cancer while limiting activation of those pathways leading to the toxic effects [77]. Evidence for the feasibility of this concept is mounting. First, non-traditional AhR ligands have been identified that are capable of activating the receptor without association with the ligand binding pocket [35]. Second, similar to steroid hormone receptors [78], different ligands induce unique conformational changes in the AhR that facilitate interaction with different transcriptional cofactors [79]. On a functional level, the AhR agonists I3C, DIM and a series of DIM analogs were shown to effectively inhibit the growth of estrogen-dependent and independent breast cancer cells and tumors in a manner comparable to TCDD [67-70,72,80]. Structure-activity studies using the DIM analogs showed that specific methyl and dihalo-substitutions could further enhance the anticancer effects above those substantial effects seen for DIM alone [67,68,80]. AhR agonists can also be optimized for tissue-selective responses. The AhR agonist 6-methyl-1,3,8-trichlorodibenzofuran (6-MCDF) was found to synergize with tamoxifen to inhibit growth of mammary tumors in rodents while inhibiting the undesirable estrogenic effects of tamoxifen in the uterus and did not interfere with the bone-protective actions of tamoxifen in ovarectomized animals [63]. Importantly, effective doses of neither DIM nor 6-MCDF produced the adverse side effects observed with TCDD exposure [49,67,81,82].

The AhR as a Potential Therapeutic Target for ER-negative Human Breast Cancers

As put forth above, breast cancers are inherently diverse with respect to hormone and growth factor receptor expression and are typically broken down into subtypes based on ER, PR, and HER2 status. The tumor subtype can impact the growth and aggressiveness of the disease as well as predict the response to different chemotherapeutics [83, 84]. Tumors lacking expression of all 3 receptors (triple-negative) are typically the most challenging to treat due to their aggressive natures and the absence of a pliable drug target.

Unlike the available ER-targeted and growth factor-targeted therapeutics for breast cancer, AhR modulators may also have utility in prevention and treatment of triple-negative tumors. In a recent study the SERM Raloxifene, used clinically in prevention of ER-positive breast cancers, was shown to prevent growth and promote regression of triple-negative breast tumors in mice [85]. A second study soon followed that implicated an AhR-dependent mechanism; raloxifene was shown to activate the AhR, and as a consequence, induce apoptosis of triple-negative breast cancer cells with no effects on normal mammary epithelial cells [86].

Other AhR ligands have recently been identified as promising therapeutics for ER-negative breast cancers. The synthetic flavonoid aminoflavone, an AhR agonist, is currently in Phase 2 clinical trials.

Several recent studies that this agent induces DNA damage and displays anti-proliferative, cytotoxic, and apoptotic activities in multiple cancer cell types including triple-negative breast [87-90]. Finally, as mentioned earlier, the AhR agonists I3C, DIM and a series of DIM analogs were shown to effectively inhibit the growth and invasiveness of ER-negative breast cancer cells and tumors [65,70,72,80]. Thus, it appears that the AhR hold tremendous potential as a pliable drug target for treatment of estrogen-independent breast cancers.

Summary and Significance

The current statistics associated with breast cancer reflect both its importance in women's health and the progress we have made towards understanding and treating the disease. The fact that the incidence and mortality of breast cancer has not declined at the same rate as other major causes of death speaks to the unmet need for both new therapeutic targets and approaches. A series of mechanistically distinct therapeutics are currently in clinical use for breast cancer, including those targeting estrogen and growth factor signaling pathways. However, it has become clear that breast cancer is a heterogeneous disease. Each cancer has its own molecular and biochemical makeup and, consequently, there exists no single drug or drug class that will be sufficient to effectively eliminate the disease in all afflicted individuals. This realization has continued to fuel efforts aimed at further molecular characterization of the disease, and these efforts have led to identification of potential and promising new drug targets such as the AhR.

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